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Effect of Dietary Calorie Levels on Reproduction and Physical Activity of Drosophila Melanogaster

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EFFECT OF DIETARY CALORIE LEVELS ON REPRODUCTION AND PHYSICAL ACTIVITY OF *Drosophila melanogaster*

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EFFECTS OF DIETARY CALORIE LEVELS ON REPRODUCTION AND PHYSICAL ACTIVITY OF *Drosophila melanogaster*

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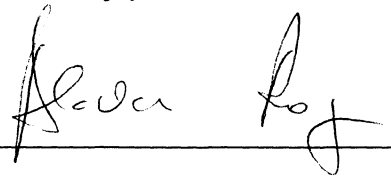
Major Advisor 

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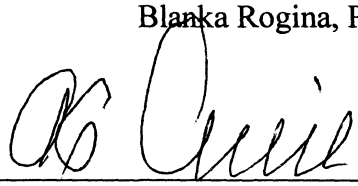
Associate Advisor 

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This thesis is dedicated to my parents and teachers

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INTRODUCTION

Aging

Among many attempts to define aging, it has been described as an organic process of irreversible and continuous deterioration of the organism's performance, which ultimately results in the death. Each species has a characteristic maximum life span. For humans it is close to 115 years. In contrast average life span (the age at which half of the population is still alive) can vary a lot. Average life expectancy for US residents in 1901 was 49 years and it increased to a record 77.6 years according to a preliminary report by CDC's National center for health statistics. (1) This increase can be attributed to better hygiene and various advances in the field of medicine.

Since times, numerous scientists have attempted to study factors and predisposing conditions which may increase the average and the maximal life span of an individual. Understanding the complex process of aging will not only benefit mankind by increasing the maximal productive quality life span but it will also provide an insight into the complex maze of biomedical processes which are interlinked and interdependent.

Studying aging can be helpful in understanding the underlying mechanism at the cellular and molecular levels which can give an insight into the various biochemical reactions taking place as a part of aging process. This might enhance our knowledge of senescence and may help unravel the methods to improve the functional quality of life during the later part of life span. Unraveling the

physiological process of aging may also help to study the pathological conditions, which mimic accelerated aging.

Theories of Aging and Longevity

Evolutionary theories of aging and longevity are the theories that make an attempt to explain the differences in observed aging rates and longevity records across different biological species.

The appeal for understanding the biological evolution of aging and lifespan comes from observations of the life cycles of some biological species like, a bamboo plant reproduces asexually for about 100 years, forming a dense plant. Then in one season all of the plants flower simultaneously, reproduce sexually, and die (Keeley and Bond, 1999) This and other similar observations of self destructive and suicidal life cycles of species like pacific salmon (Patnaik et al., 1994) have promoted the thought that sexual reproduction may have a negative effect on the species lifespan.

It has been suggested that in addition to mutation and selection, the reproductive cost may play as a trade-offs between different traits of organisms and contribute to the evolution of species aging and longevity. The evolutionary theories of aging are closely related to the genetics of aging because biological evolution is possible only for heritable manifestations of aging. (Gavrilov and Gavrilova, 2002)

The theory of programmed death

August Weismann (1834–1914), was one of the first biologists who used evolutionary arguments to explain aging. His initial idea was that there exists a specific death-mechanism designed by natural selection to eliminate the old, and therefore worn-out, members of a population (Weismann A)

The free radical theory

Denham Harman proposed the **free radical theory** of aging in 1954. Harman tested it by administering dietary antioxidants to various strains of mice and showed that radioprotective compounds prolonged the median life span of mice (Harman, 1956). Many dietary antioxidant studies have followed over the years, confirming that free radicals play an important role in the aging process. A free radical is a molecule carrying an unpaired electron. Free radicals are extremely reactive and will seek out and acquire an electron from surrounding environment. In the process of acquiring an electron, the free radical attaches itself to another molecule; thereby converting these previously stable molecules into free radicals.

Such free radical molecules are capable of damaging any biomolecule, including proteins, sugars, fatty acids and nucleic acids. Theories of aging can be broadly classified into two categories: Stochastic theories and Pleiotropic theories. Stochastic theories argue that aging is caused by random chemical insults at the molecular level, such as damage from free radicals. Pleiotropic theories claim that aging is genetically programmed and therefore essentially unchangeable. Free

Radical theory has been further evolved and studied as the **oxidative stress hypothesis.** The main premise of this hypothesis is that aging is a continuation of development and is influenced by genetically programmed phenomena.

Oxidative stress is one of the factors, which has been shown to govern changes in gene expression during differentiation and may cause alterations in gene expression during aging.

The oxidative stress hypothesis has been widely accepted to explain the biochemical changes and effects taking place in an aging cell and organism. (Sohal and Weindruch, 1996) {Beckman, 1998 #1056} ; {Finkel, 2000 #1033} ; (Sohal et al., 2002) . It is proposed that highly reactive oxygen radicals are generated as the byproducts of aerobic respiration. In the event of an imbalance between the generation of these reactive oxygen species (ROS) and their detoxification various oxygen radicals escape and damage a range of biomolecules. This damage is thought to induce further damages at the cellular level, resulting in accelerated decline in physiological functions and increased risk of cell death. Accordingly, aging is suggested to be a result of cumulative oxidative damage generated by reactive oxygen species (ROS) produced during respiration.

Oxidative damage to DNA, RNA, protein, and lipids has been demonstrated to occur during aging. This damage may be limiting the life span. In another study simultaneous over expression of the enzyme superoxide dismutase (SOD) and catalase (cat), has been observed to reduce ROS production and extend the life span of *Drosophila* (Sohal et al., 2002) .

Calorie intake – lifespan relationship

Calorie restriction with adequate nutrition has been now known for over 70 years to increase both the average and maximal life span of rodents. The increase in life span of up to 50% results from the limitation of total calories derived from carbohydrates, fats, or proteins to a level 25%-60% below that of control animals. Recently it has been shown that the calorie-restriction extends life span across distant species including yeast, rotifers, spiders, worms, fish, mice, and rats. In studies with primates on calorie restriction many beneficial effects are periodically reported.

Caloric restriction has been observed to delay a wide spectrum of diseases in different experimental animals such as kidney disease, a variety of neoplasias, autoimmune disease, and diabetes. CR reduces age-associated neuronal loss in most mouse models of neurodegenerative disorders such as Parkinson's disease or Alzheimer's disease (Guarente, 2005). The CR regimen also prevents age-associated declines in psychomotor and spatial memory tasks and improves the ability for self-repair.

The effects of calorie restriction in extending the survival and delay in the process of aging in rodents, and other organisms has been expected to be a result of slowing in the rate of accumulation of age-related oxidative stress. This conclusion can be based on the increase in amount of (ROS) the oxidation products of proteins, lipids and DNA in tissues with aging. It has been proposed that CR feeding slows the rate of accumulation of ROS as mitochondria in these animals have a lower rate

of superoxide generation when compared with mitochondria from control animals fed on high caloric diet. This damage may be limiting the life span. In another study simultaneous over expression of the enzyme superoxide dismutase (SOD) and catalase (cat), has been observed to reduce ROS production and extend the life span of *Drosophila* (Sohal et al., 2002).

It is also observed that oxidative damage is reduced in CR animals. This reduction in the production of ROS could possibly be a consequence of slowed metabolism, though present studies give conflicting results. Another possibility could be a more efficient transport of electrons through the respiratory cycle or the ability of an organism to damage repair.

"Syndrome X" has been identified in humans by Gerald Reaven, which consists of the combined presence of insulin resistance and glucose intolerance, obesity, blood fat abnormalities (including free radical-oxidized cholesterol), and hypertension (J. Chellam, B. Berkson & M. Smith.). These "Syndrome X" parameters are known to be reduced in animals undergoing caloric restriction (Sohal and Weindruch, 1996). Sir2, a histone deacetylase has been implicated as a major player in mediating beneficial effect of caloric restriction. Sir2 mediates changes in metabolism increasing stress resistance and decreasing apoptosis

Diet and reproduction

Reproductive activity has been considered to be a measure of female fertility and male virility of an organism. In female fruit flies the level of viable egg production is an acceptable measure of reproductive activity. Female *Drosophila*

melanogaster exhibit multiple mating during the life time and they have the ability to store sperms passed during mating. Various selective factors have been suggested to favor multiple mating like replenishment of sperm, fertility insurance. Sperm competition, seminal feeding and genetic divergence among progeny (Chapman and Partridge, 1996)

Reproductive activity has been shown to exhibit a major influence on the life span of female fruit flies. In previous studies done with female *Drosophila melanogaster* it has been seen that females, which are exposed to increased amount of mating, have shorter life spans (Chapman et al., 1993).

In life span studies performed on females, which were mated with males, which transferred sperm, and spermless males, the life span was not different. Thus cost of mating cannot be attributed to passing of sperm during mating (Chapman et al., 1995). It has also been seen that seminal fluid products from main cells of accessory glands like the accessory gland proteins such as sex peptides eg.Acp70A has been shown to be responsible for the cost of mating seen in female flies (Wigby and Chapman, 2005)

The products of main cells of male accessory glands are responsible for increases levels of egg production. Flies have been observed to store these products for days after mating and this result in continuously increased levels of egg production after mating (Chapman et al., 1995) . This cost of mating observed in the female, fruit flies have been studied by comparing the lifespans of mated v/s virgin flies. Virgin flies have been observed to have a longer life span and overall similar or even higher life time egg production, partly because of their longer life

span. Thus this cost of reproduction can not solely be explained on the basis of physiologic processes taking place during egg production. (Partridge et al., 1986).

In studies done with intermittently and continuously mated fruit flies maintained at different levels of nutrition, it was observed that age-specific and life time egg production increased with the increase in the nutritional content of the food. Preliminary data from our laboratory shows that the female fruit flies on the highest caloric levels exhibit a cost of mating by shortened life span. At the highest food level reduced lifespan lead to a significant cost of mating for egg production. The preliminary data from our study of egg production of virgin female fruit flies at different nutritional levels is very promising as it eliminates the effect of mating and it also gives a larger range of nutritional levels to compare, as we have used six different food levels.

Diet and physical activity

Physical activity has been shown to impact the life span of housefly in previous studies done with flies which had their wings removed. Similar results are also seen in flies maintained in small containers, which inhibited the physical activity of flies, resulting in the extension of the life span. These houseflies restricted to small space lived twice as long as the flies housed in large cages. Such effects have lead to the belief that the life-span of flies is inversely proportional to the metabolic activity of the fly, which can be altered by varying the levels of physical activity undertaken by the organism (Agarwal and Sohal, 1994)

In a recent study examining the relationship between longevity and metabolism in a large number of recombinant inbred *Drosophila melanogaster* lines, the inverse relationship between longevity and metabolic rate was not observed. This study describes no correlation in these lines between metabolic rate and longevity, indicating that the ability to maintain a normal metabolic rate and have extended longevity may apply to *D. melanogaster* in general. This study used long-term, flow-through metabolic rate measurements and closed system respirometry and examined the effects of variables such as time of day, feeding state, fly density, mobility of the flies, and nitrogen knockout on *D. melanogaster* metabolic rate. The CO_2 production was estimated in individual flies reflecting metabolic rates of flies under the conditions used for longevity assays (Van Voorhies et al., 2004).

It has been observed that the fruit flies exhibit daily rhythms of physiology and behavior, which are precisely timed by an endogenous circadian clock. This rhythm has been observed to be two separate bouts of morning and evening activity. It has been seen that the timing of morning and evening activity in Drosophila derives from two distinct groups of circadian neurons, the morning activity is derived from the ventral lateral neurons that express the neuropeptide PDF, and evening activity from another group of cells, including the dorsal lateral neurons (Stoleru et al., 2004).

OBJECTIVE

To study the effect of various caloric levels on the fertility and the physical activity of *Drosophila melanogaster*

1. To study and compare the number of eggs produced by virgin female flies of *Canton-S5* strain on various foods of different caloric content
2. To study and compare the physical activity of virgin flies maintained on various foods of different caloric content
3. To study the effects on physical activity of flies switched from low caloric diet to high caloric diet and vice-versa at various stages in their life

HYPOTHESIS

Increasing amount of calories in the diet proportionally increases the reproductive activity and inversely affects the physical activity of *Drosophila melanogaster*

MATERIAL AND METHODS

Study model

Drosophila melanogaster is used as the study model for this research. Preliminary study in our laboratory show that fruit flies consume similar amount of food, thus effects of increased caloric content in the food can be studied directly as the total intake of food does not differ considerably. The fruit fly, *Drosophila melanogaster* is one of the most common genetic model organisms used for studying aging. The primary benefits of using Drosophila for lifespan study is relatively short life span (up to 2 months at 25°C), which is much shorter than rodents and primates (Miquel J, Lundgren PR, Bensch KG, Atlan H. 1976; Helfand & Rogina 2003). It is also easy to maintain large stocks with inexpensive housing and food. Also, stocks containing altered genes, flies with various established mutations and genetic alterations are available in our lab and most of the Drosophila genome has been sequenced, and the many fly genes have homologues in mammals.

Fly stocks

The Canton-S5 strain used for these experiments is the standard wild-type background employed in our laboratory and has been maintained in our laboratory as an inbred stock for many years. This stock is maintained at 25 C in a humidity controlled incubator (Percival Scientific) on a 12 hour light:dark cycle. A stock culture of approximately 50 vials (50 mm, plastic) each containing 15 males and 15 females was continuously maintained at all times. Prior to the initiation of the

experiments, the stock population was expanded and the newly eclosed flies were introduced and maintained in different food levels required for this study.

Dietary calorie content of Drosophila food

Seven different food types distinguished by the caloric content of each were used in this study. These different food levels are standardized as 1.0N being the food that has 100 g/L of sucrose (MP Biomedicals, Inc), 100 g/L of brewer's yeast (MP Biomedicals, Inc) and 20 g/L of agar. The food components are mixed and autoclaved for 20 minutes. The food is cooled to a temperature of 65° Celsius and 3ml of food is poured in glass vials for the activity monitoring. 0.1N, 0.2N, 0.5N, 1.0N, and 3.0N food level included in the study evaluating the effects of these various dietary caloric contents on the resultant physical activity.

For the food prepared for the fertility experiment, mold inhibitor Tegosept (methyl paraben) was added to a final concentration of 2.3 g/l after the cooling to 65° Celsius of the autoclaved food, and then the 10 ml of food is poured in plastic vials. Tegosept was added only after cooling of the food to prevent denaturation and degradation of the chemical compound. Following pouring of food in the plastic vials it was allowed to cool down to the room temperature. 0.1N, 0.5N, 1.0N, 1.5N, 2.0N, and 3.0N food levels included in the study examining the effects of these various dietary caloric contents on the reproductive activity of flies.

Physical activity monitors

Drosophila activity monitor is manufactured by Trikinetics Inc., USA.

The ***Drosophila*** **population monitor** is built around a standard 25 mm diameter glass vial. A population of 10 males or females is introduced to the vial with appropriate food level. The vial is closed by sponge and placed in locomotor recording chamber. As the flies walk back and forth along the walls of the tube, they interrupt the infrared beam rings, which cross in 3 places along its length. The infrared beam rings form narrow planes of invisible light which cross the tube, perpendicular to its long axis. A fly which moves anywhere within the plane of such a beam, either on the wall or in the middle, will be detected and counted as an activity event. These beam interruptions are detected, counted and reported periodically to the host computer over the DAM System wiring network. The data was then collected and plotted using excel and Kaleidagraph software.

For each food level four replicate of ten males or females were monitored for their activity throughout the life span. The flies and food was changed every two days. The beam passes once every 10 minutes through the vial and a recording is saved. Activity monitors were housed in incubators maintained at 25°C on a twelve hour light: dark cycle maintained throughout this experiment.

Availability & location of research facilities

These experiments were conducted in the laboratories of Dr. Blanka Rogina and Dr. Stephen L. Helfand located in the rooms E-3029, L-2004 and L-2008 at the University of Connecticut Health Center. I had full access to all lab equipments and materials to complete these experiments. Various food levels ranging from 0.1N to 3.0 were mixed, autoclaved and poured by Diana Schwarz, Lab assistant.

Methods

20 vials with one virgin *Canton-S5* female fly each were kept from the day of eclosion on the appropriate dietary level. Vials were passed every day to a new vial when the number of eggs counted using a simple microscope.

Statistical analyses

The data was saved using the DAMS software (Trikinets Inc.) then the files were plotted on excel worksheet (Microsoft Inc.) The average daily activity was calculated and plotted using the Kaleidagraph software. The standard error in the mean activity and other analysis were performed using the Kaliedagraph software

RESULTS

Effects of dietary calorie levels on egg production

Virgin female flies maintained on different dietary caloric levels exhibit difference in the number of eggs produced. In a comparison with mated flies (Fig.3) the virgin female flies appear to have a longer life span.

The virgin female flies appear to be producing much lesser number of eggs than the mated flies (Fig.4)

Virgins flies exhibit life time increase in egg production with increased nutritional content, the flies maintained on highest caloric level 3.0N appear to be producing the highest number of eggs (Fig.5&6) The flies maintained on 0.5N food

level appear to have the longest life span though the life time egg production is lesser than 3.0N. Flies on 1.5N and 2.0N food level appear to be producing similar amounts of eggs. The total lifetime egg production appears to be directly proportional to the nutritional content of food.

Drosophila melanogaster also exhibit an age-dependent pattern of egg production. The largest number of eggs is produced close to Day 10 after eclosion in virgin flies (Fig.7). Thus showing a delayed increase in egg production compared to the mated flies as the peak of egg production is achieved earlier, around day 3-5 in mated flies. Flies also exhibit a reduction in the average production of eggs with increasing age.

Effects of dietary caloric levels on physical activity

The dietary caloric level appears to have a strong influence on the Physical activity of both male and female flies.

Males appear to have higher physical activity than females on the low caloric (0.5N), 1.0N and the high caloric (3.0N) food levels (Fig.10, 11, 12)

The average activity measured on 10-minute interval for first 40 days of Drosophila life span exhibit the effect of caloric content of food on the resultant physiologic functions (Physical activity). Caloric restriction with adequate nutrition (0.5N food level) appears to give maximal physical activity.

There is an age-dependent decrease in fly activity on 0.5N, 1.0N & 3.0N. This change is evident by comparing the average physical activity during the first 48 hours of life and the last 48 hours (Day 39-40) (Fig.17, 18)

There appears to be an increase in activity associated with increased age in 0.1N & 0.2N flies. This effect is evident by comparing the average physical activity during initial and latter part of life span (Fig. 19, 20)

Effects of switching the diet from low to high caloric content & Vice-versa on the Physical activity of 10 day old *Drosophila melanogaster*

The flies switched from high to low caloric diet show a gradual increase in the physical activity. This change can be appreciated as early as one day after switching the diet from 3.0N to 0.5N food level (Fig.21)

Female flies switched from high to low diet show a delayed response in increasing the physical activity (Fig.22)

Flies when switched from low to high caloric food or from the high to low caloric diet still appear to be maintaining the memory of physical activity corresponding to the physical activity of the diet prior to the switch. When flies are switched from high to low caloric food there appears to be an increase in the physical activity of the flies, but the new average activity after the switch is still lower than the flies continuously maintained on low caloric diet from day 0 (Fig.25, 26)

DISCUSSION

The dietary calorie restriction has been undoubtedly imparting beneficial effects by extending the life span of a variety of species including the fruit flies. The Dietary caloric restriction has been suggestive to be a diet with adequate amount of nutritional value necessary for the total well being of the organism. Total well-being, or the concept of fitness address an optimal performance level of an individual where the individual is able to satisfactorily perform the usual physical activities and is satisfactorily reproductively active. The level of calorie content in diet appears to have a significant role in affecting the longevity, mobility and the rate of egg production.

A dietary calorie level which can optimize and impart a balance to such physiological functions will help attain a state of fitness for an organism where the organism can maintain itself and benefit from the diet and perform well on the parameters of longevity, mobility and reproductive activity.

During my study of the physical activity of fruit flies, which were maintained at different levels of dietary calorie levels it was evident that the female flies had lower levels of physical activity than their male counterparts. This effect appears to be a result of increased weight and the physiological function of egg production seen in the female flies. It was observed that there was a proportional reduction in the physical activity and a proportional increase in the rate of egg production in the female flies with increase in the dietary calorie levels. It will be further of interest to study the effect of various contents of the diet i.e. study the

effect of carbohydrate content (sucrose) v/s the protein content (yeast) in diet and study the resultant effect on the life span, physical activity and the rate of egg production.

CONCLUSION

Dietary caloric levels have a profound effect on the egg production of female Drosophila melanogaster irrespective of their mating status. Higher caloric diet increases the number of eggs produced in both mated and virgin flies.

Dietary calorie intake effects physical activity of both males & females flies. Flies on caloric restricted diet have higher levels of physical activity. Males have higher levels of Physical activity on all caloric levels compared to female fruit flies.

Switching of diet results in reversal of physiological function (physical activity) studied in this study. The flies when switched from high caloric diet to low caloric diet show an increase in their physical activity.

Table 1

Composition of fly food used

Food Type	Sucrose	Brewer's Yeast	Agar
1.0N	100g/L	100g/L	20g/L

Table 2

List of fly food used for different experiments

Reproductive Activity	0.1N	0.5N	1.0N	1.5N	2.0N	3.0N
Physical Activity	0.1N	0.2N	0.5N	1.0N	3.0N	

Figure 1

The effects of various caloric levels in diet on the life span of male *Drosophila melanogaster* The dietary caloric levels described are 0.5N, 1.0N, 1.5N, 3.0N. The flies on lower caloric levels have longer life span. Data from Dr.Tyson Bross, PhD.2005, Univ. of Connecticut

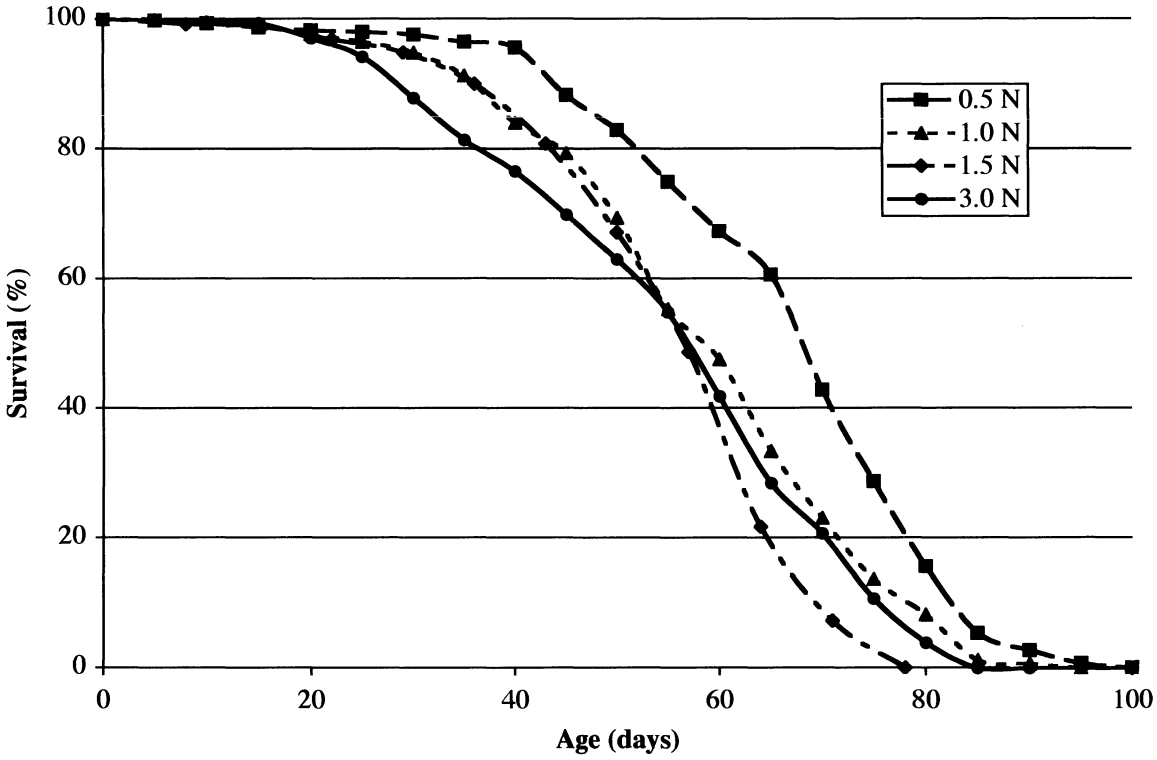


Figure 2

The effects of various dietary caloric levels on the life span of female *Drosophila melanogaster* The dietary caloric levels described are 0.5N, 1.0N, 1.5N, 3.0N. The flies on lower caloric levels have longer life span. Data from Dr.Tyson Bross, PhD.2005, Univ. of Connecticut

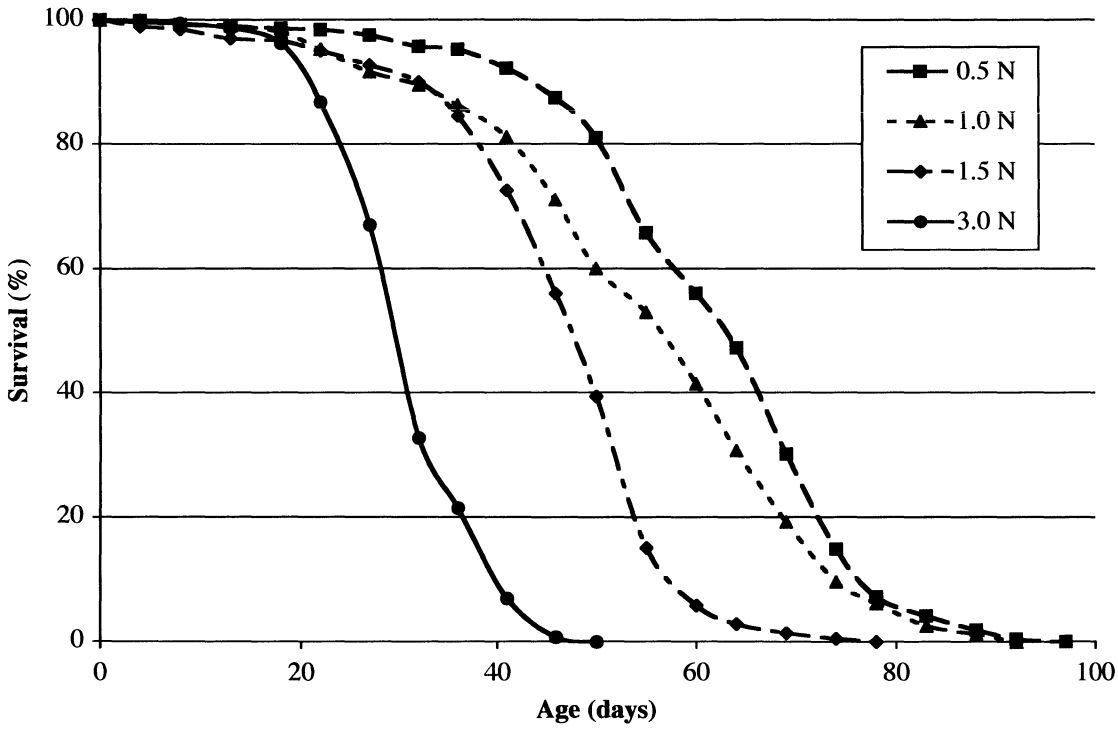


Figure 3

Comparison of the life span of mated vs. virgin female fruit flies. Virgin flies have a longer life span than the mated flies. Unpublished data from Dr.Blanka Rogina, University of Connecticut

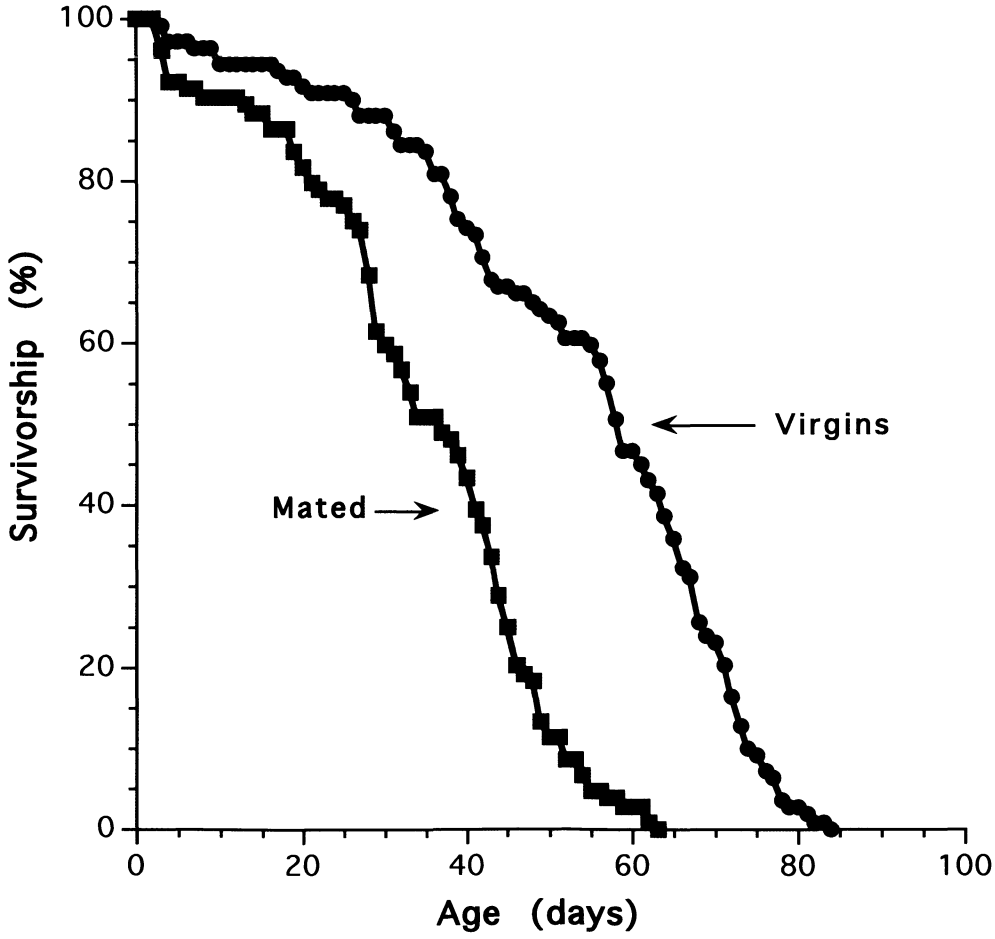


Figure 4

The cumulative egg production of mated fruit flies maintained on 0.1N, 1.0N and 3.0N food levels. The flies on 3.0N produce largest amount of eggs. Unpublished data from Dr.Blanka Rogina, University of Connecticut

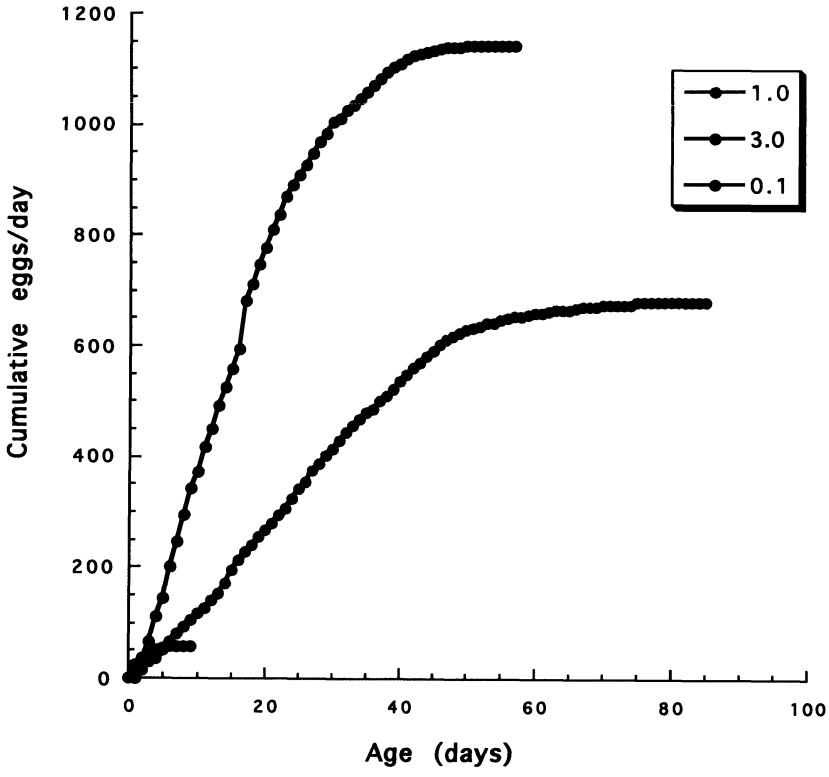


Figure 5

Effects of caloric intake on average egg production of 20 virgin female fruit flies plotted with daily standard deviation in egg production. Flies maintained on 3.0N have one fold increase in egg production compared to flies on 0.5N food level

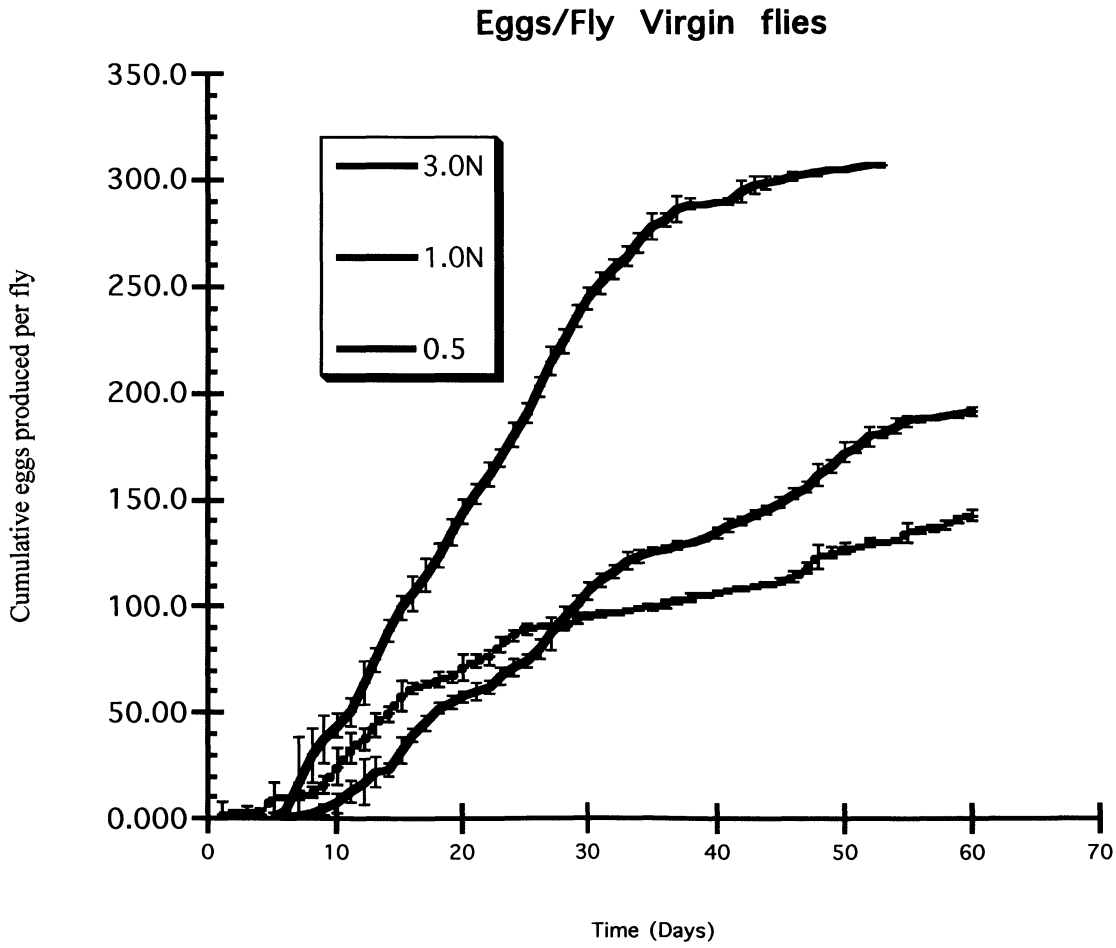


Figure 6

Effects of caloric intake on average egg production of 20 virgin female fruit flies studied on 6 different caloric levels. 0.1N, 0.5N, 1.0N, 1.5N, 2.0N, 3.0N Flies maintained on 3.0N produce largest number of eggs. 2.0N and 1.5N produce similar but lesser eggs than 3.0N followed by 1.0N and 0.5N. The flies on 0.1N produce minimal number of eggs.

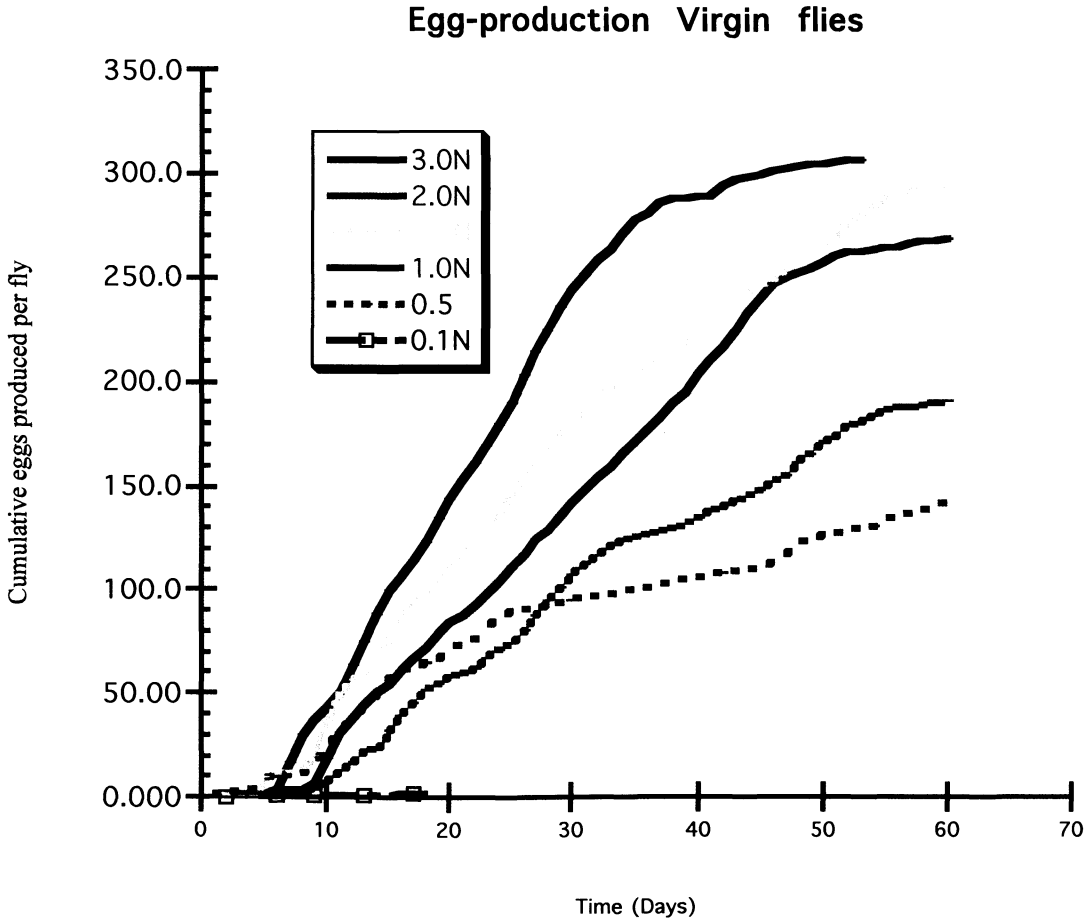


Figure 7

Average daily egg production by virgin fruit flies on 0.1N, 0.5N, 1.0N, 1.5N, 2.0N, and 3.0N food levels. The flies exhibit a peak in egg production on day 10

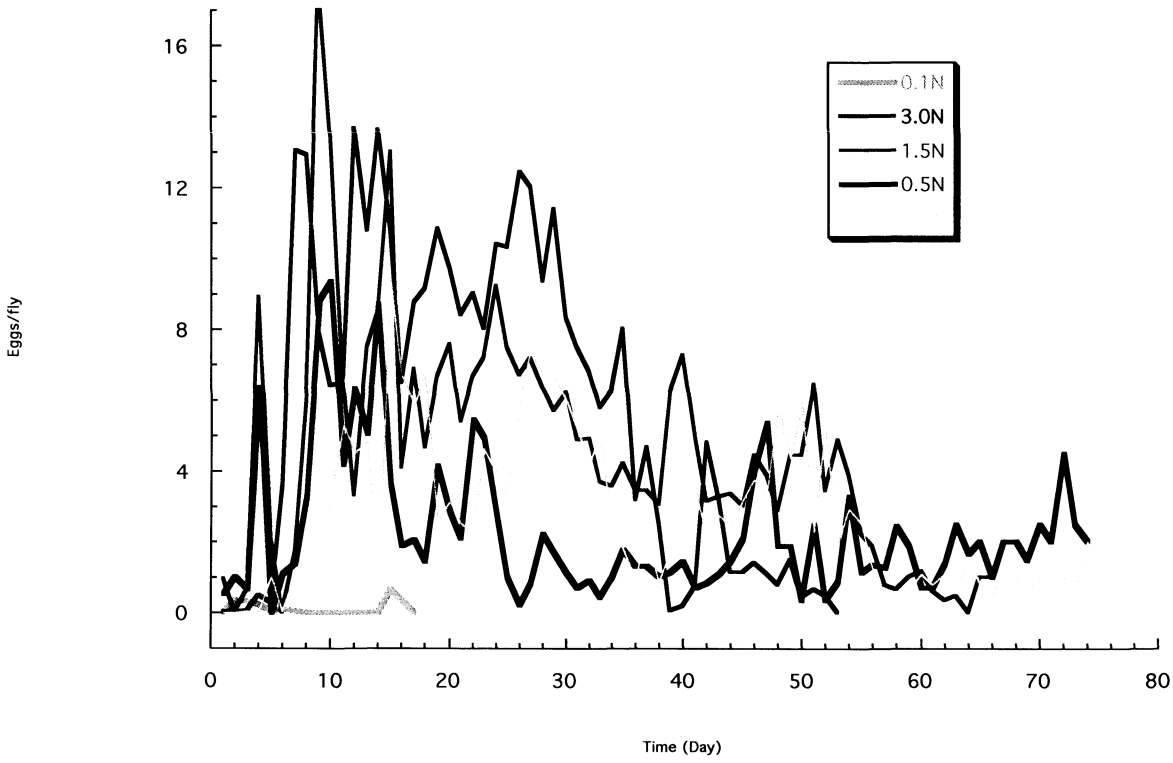


Figure 8

Total number of eggs produced by virgin female fruit flies on 0.1N, 0.5N, 1.0N, 1.5N, 2.0N, and 3.0N food levels

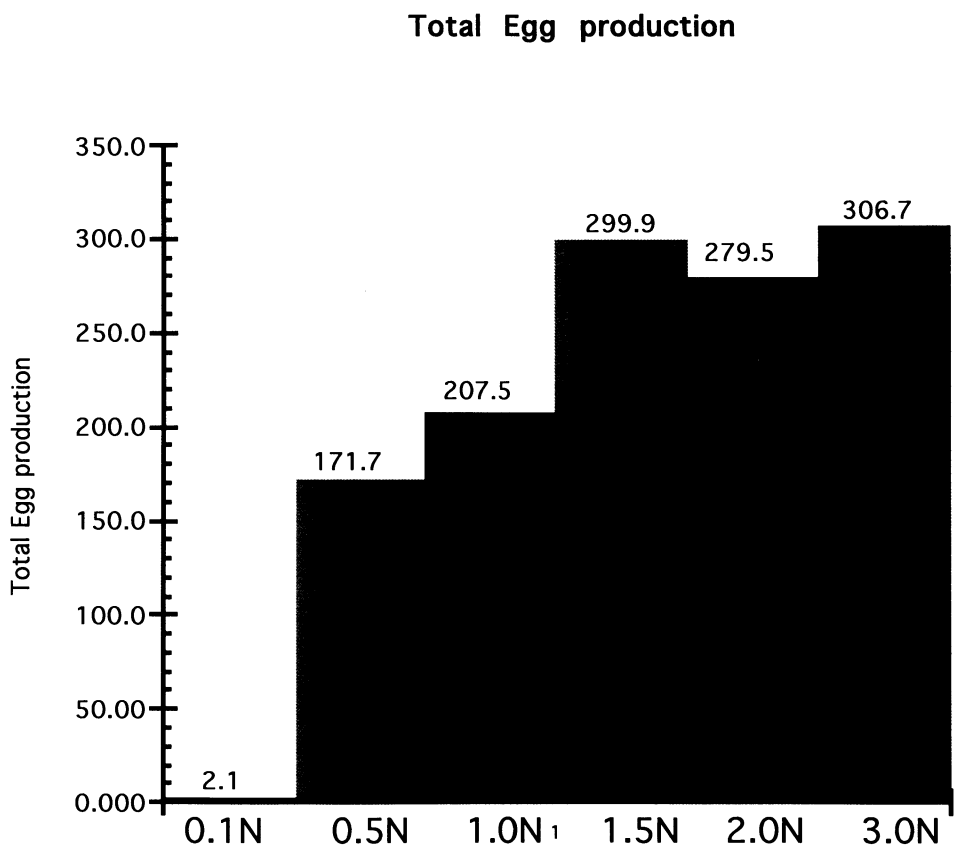


Figure 9

Average daily physical activity of male fruit flies on 0.5N, 1.0N and 0.5N food levels recorded every 10 minutes over a period of 40 days. The flies on 0.5N have highest activity followed by flies on 1.0N and 0.5N food levels

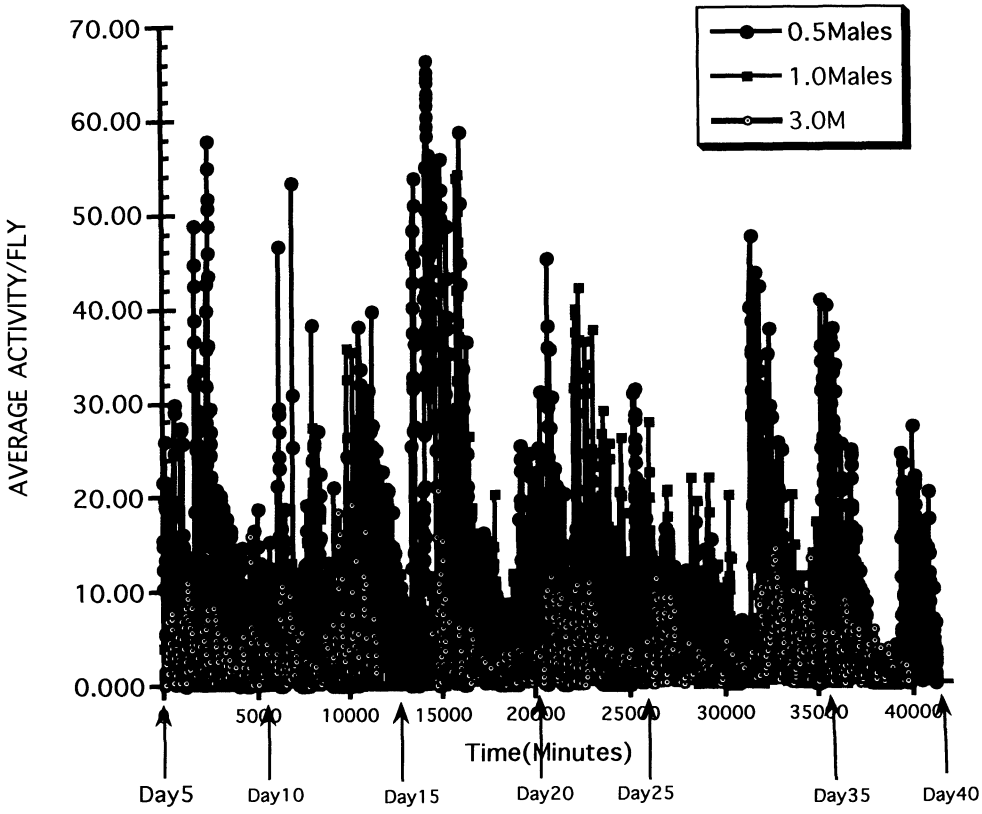


Figure 10

Comparison of average daily physical activity of male and female fruit flies on 0.5N food level recorded every 10 minutes over a period of 40 days. The male flies on 0.5N have higher activity than the females

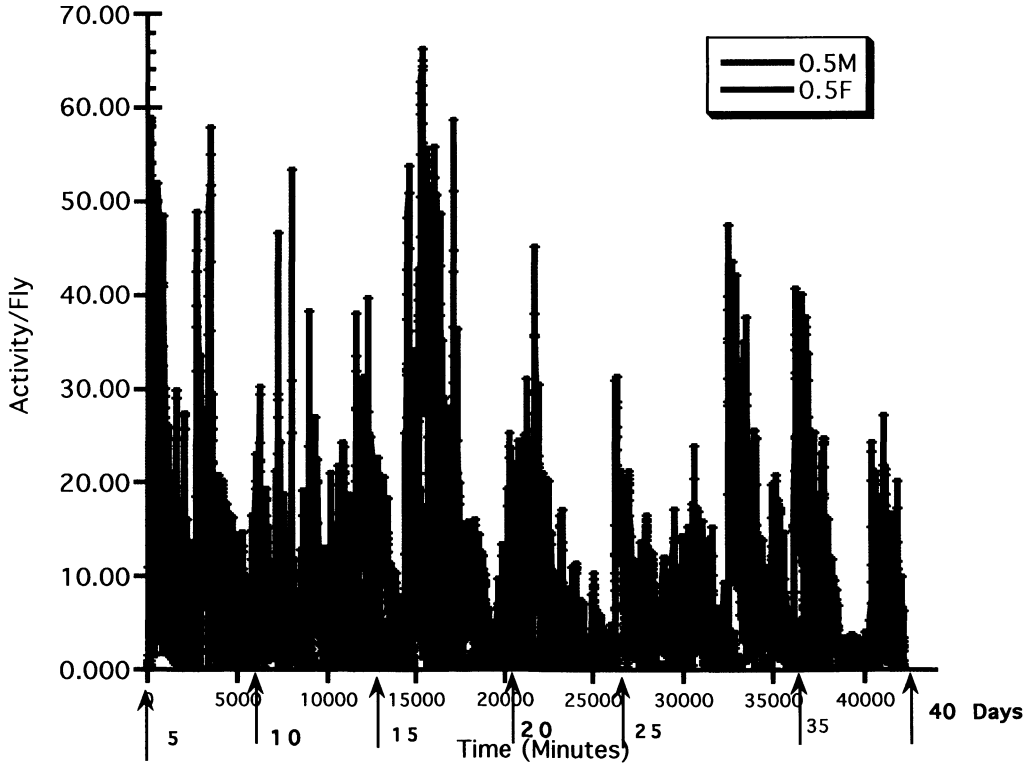


Figure 11

Comparison of average daily physical activity of male and female fruit flies on 1.0N food level recorded every 10 minutes over a period of 40 days. The male flies on 1.0N have higher activity than the females

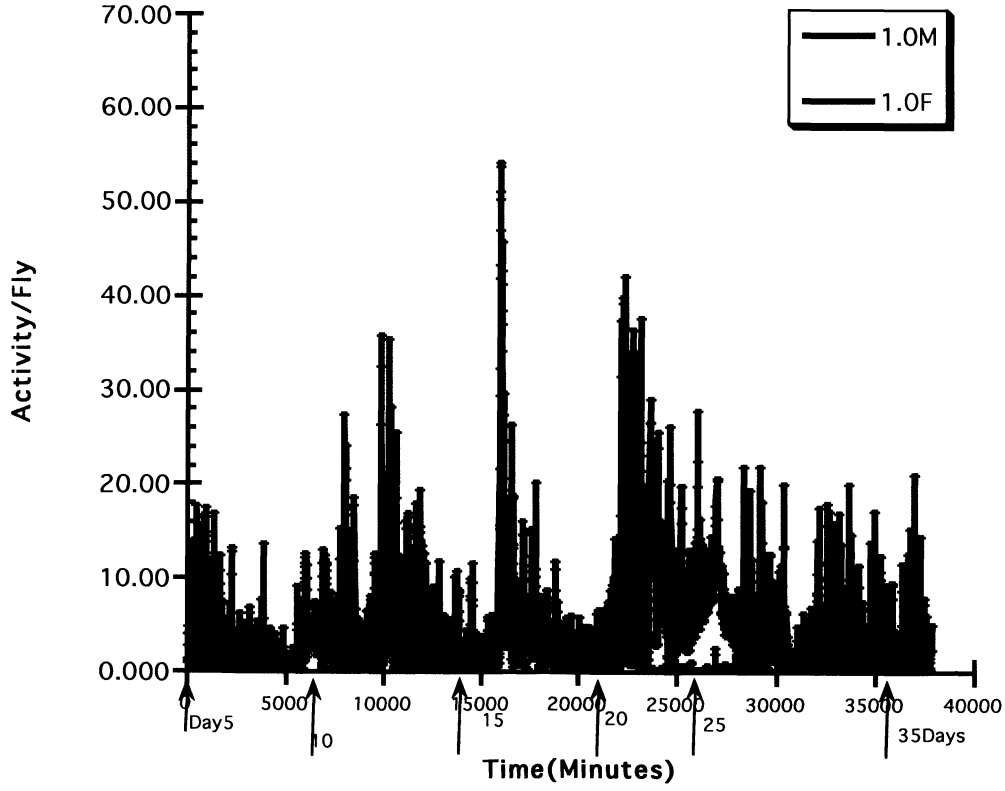


Figure 12

Comparison of averagedaily physical activity of male and female fruit flies on 3.0N food level recorded every 10 minutes over a period of 40 days. The male flies on 3.0N have higher activity than the females

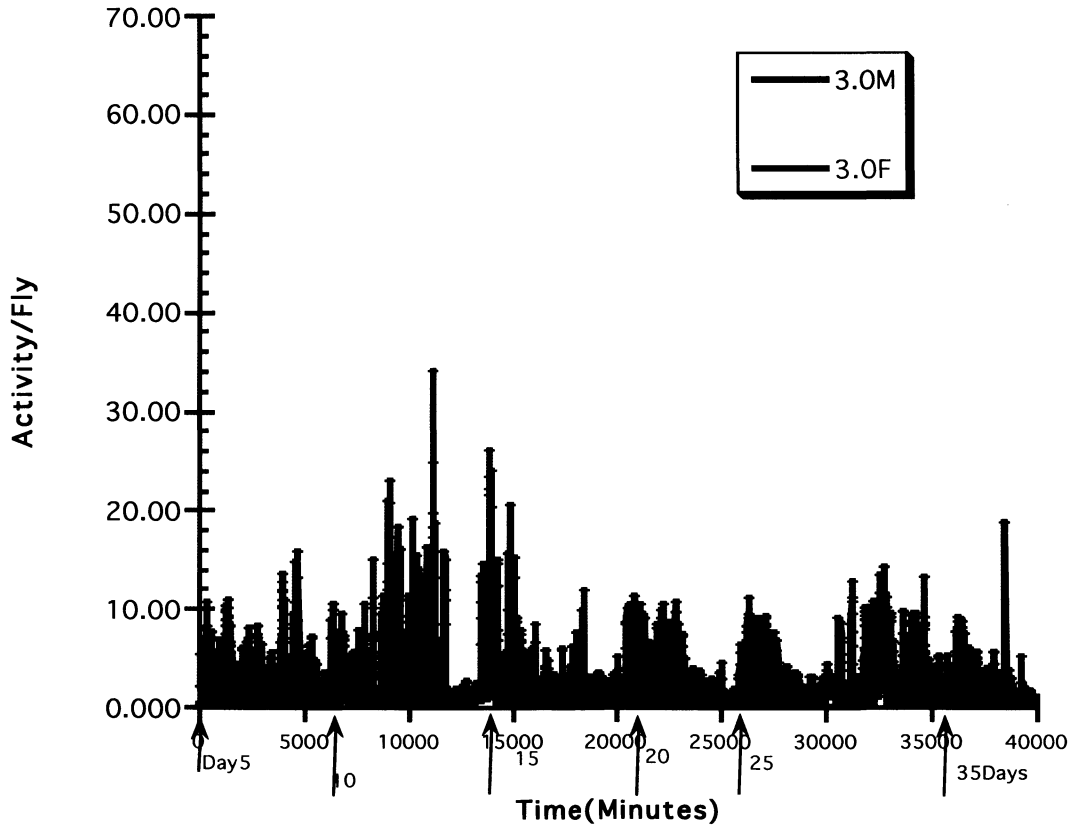


Figure 13

Comparison of average daily physical activity of male and female fruit flies on 0.1N food level recorded every 10 minutes over a period of 20 days. The male flies have higher activity than the females

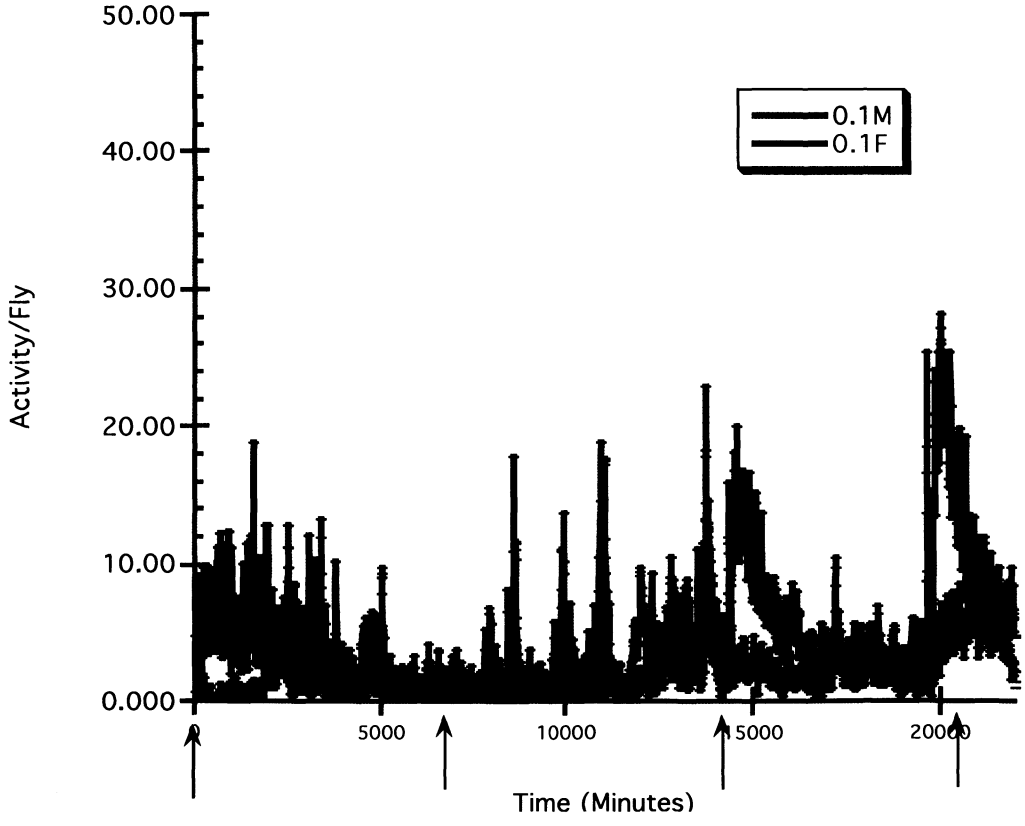


Figure 14

Comparison of average daily physical activity of male and female fruit flies on 0.2N food level recorded every 10 minutes over a period of 20 days. The male flies have higher activity than the females. The flies appear to have increase in activity with age

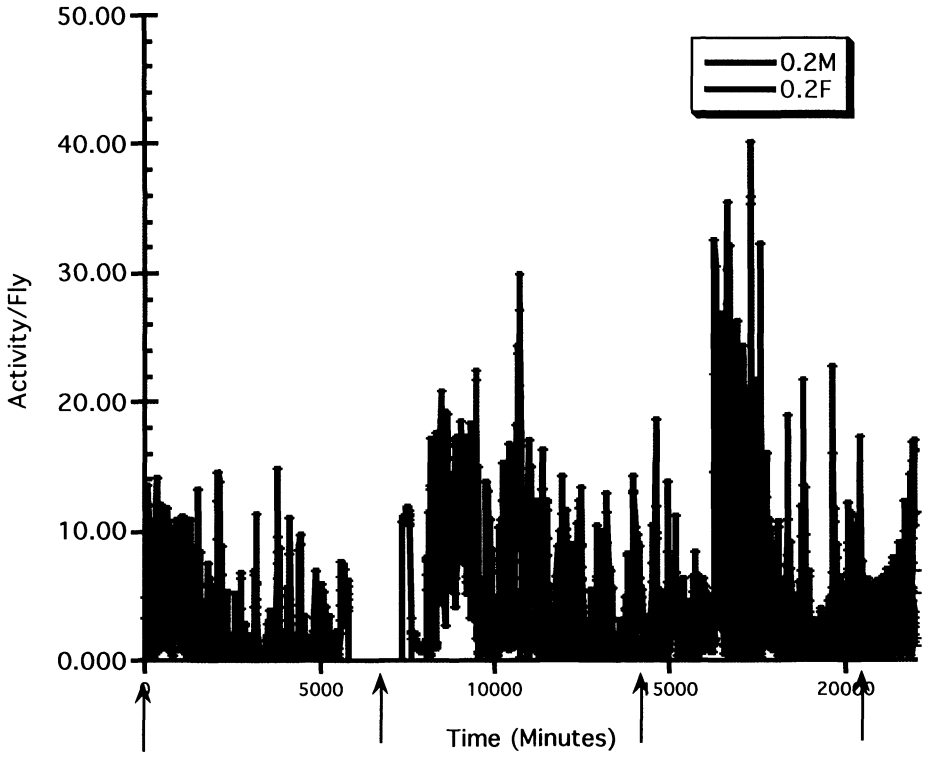


Figure 15

Mean fly activity for 5-40 day period of male fruit flies maintained on 0.1N, 0.5N, 1.0N, 1.5N, 2.0N, and 3.0N food levels plotted with their average standard errors. Flies on 0.5 have highest activity followed by flies on 1.0N, 0.2N, 1.0N and 3.0N food level

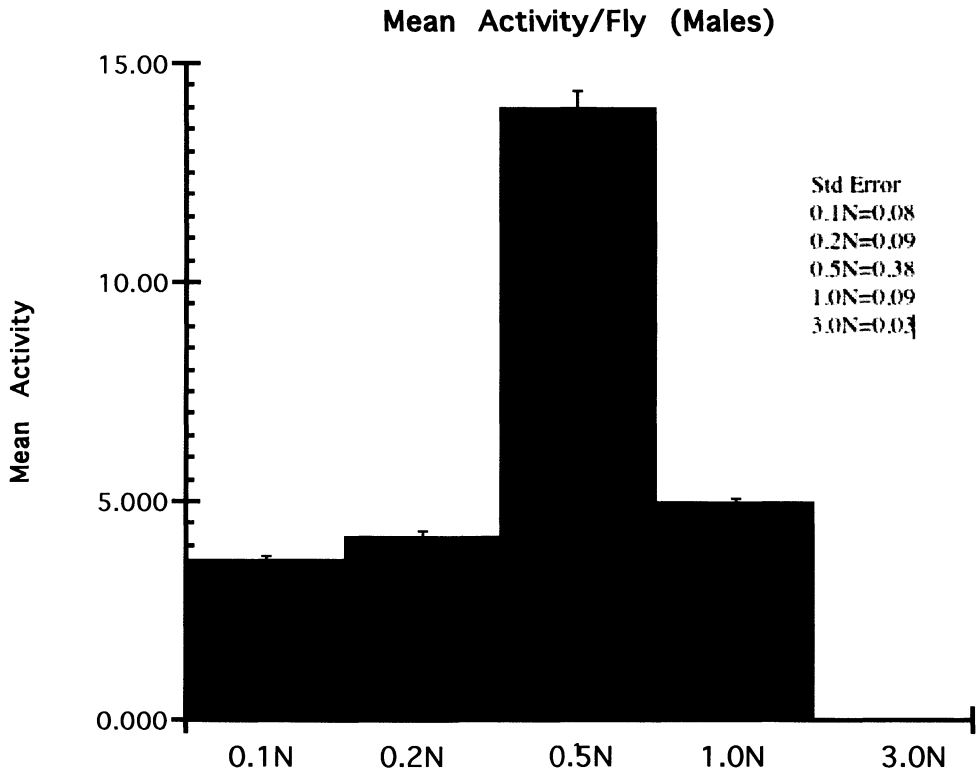


Figure 16

Mean fly activity of female fruit flies maintained on 0.1N, 0.5N, 1.0N, 1.5N, 2.0N, and 3.0N food levels plotted with their average standard errors. Flies on 0.5 have highest activity followed by flies on 0.2N, 0.1N, 1.0N and 3.0N food levels

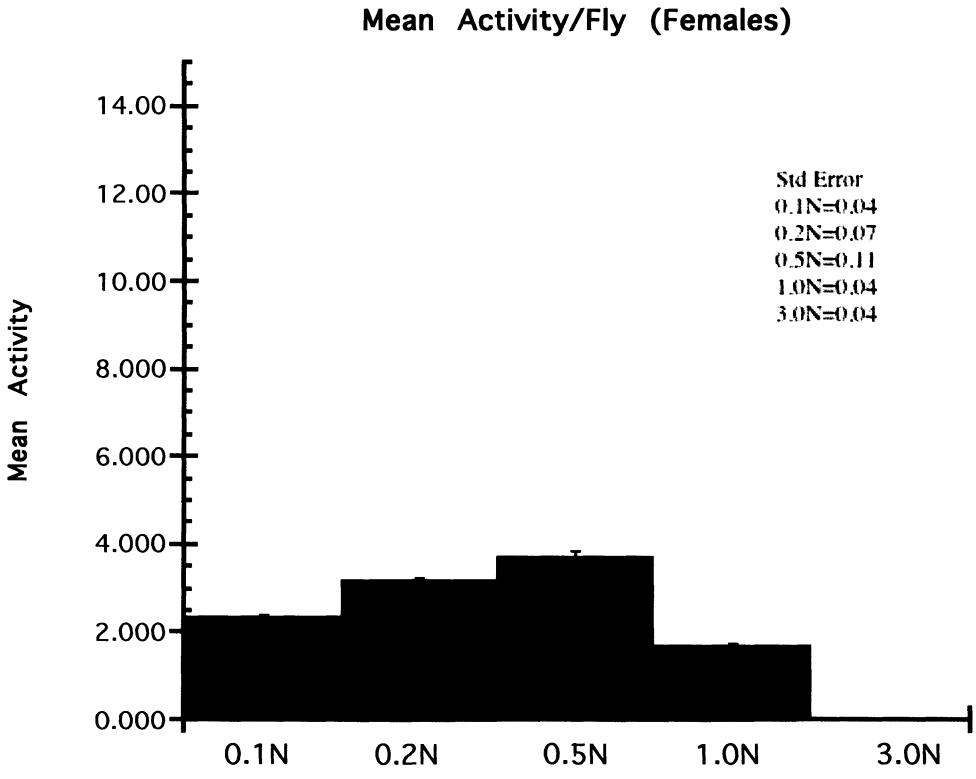


Figure 17

Age dependent changes in average mobility of flies maintained on 0.5N food level. The activity of first 48 hours compared with last 48 hours shows a relative decline in the activity

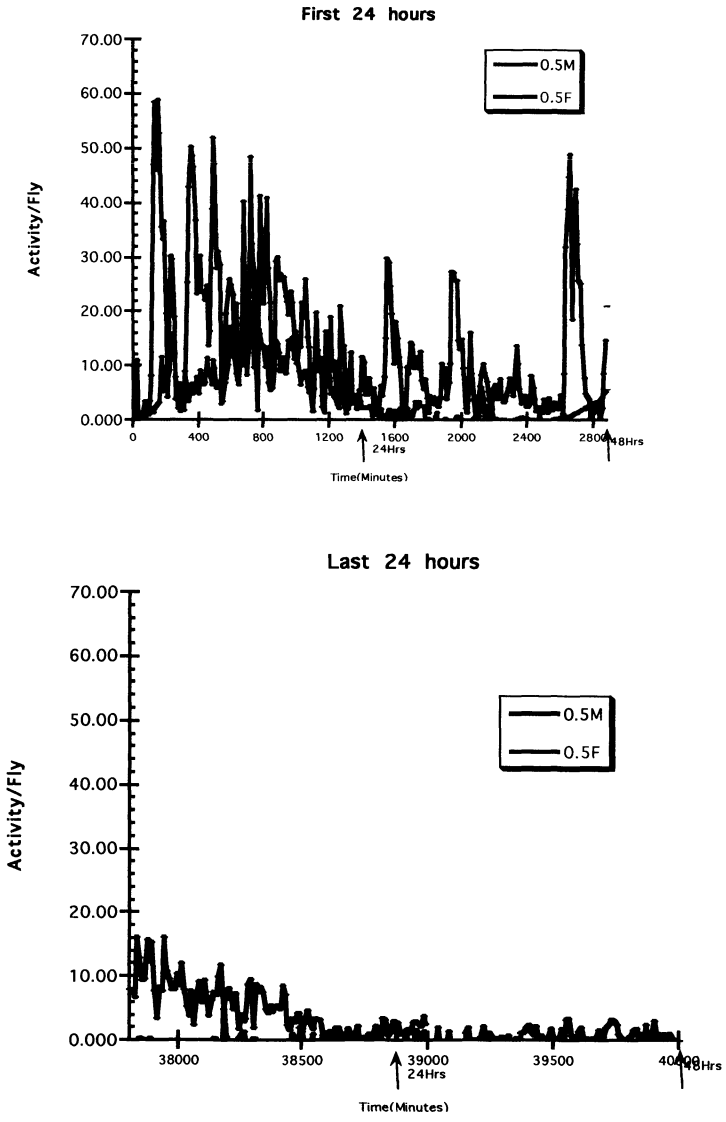


Figure 18

Age dependent changes in average mobility of flies maintained on 3.0N food level. The activity of first 48 hours compared with last 48 hours shows a relative decline in the activity

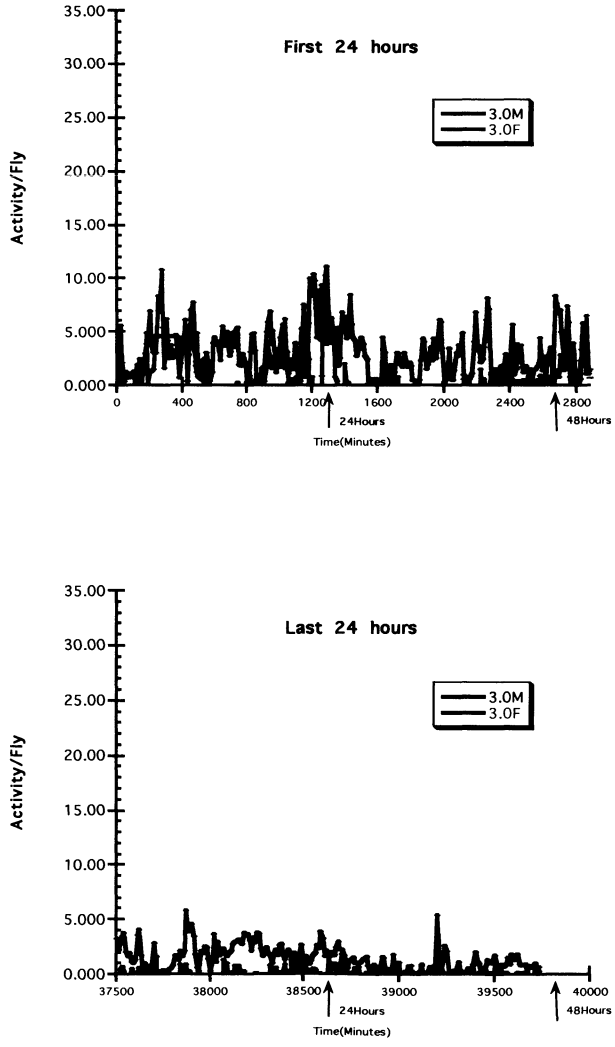
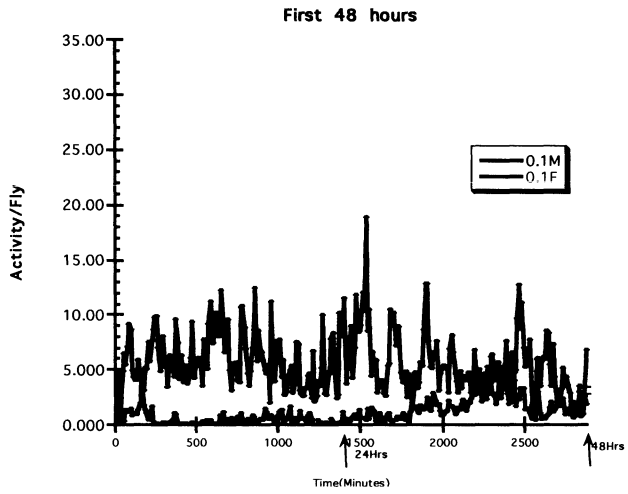


Figure 19

Age dependent changes in average mobility of flies maintained on 0.1N food level. The activity of first 48 hours compared with last 48 hours shows a relative increase in the activity just before death of the flies



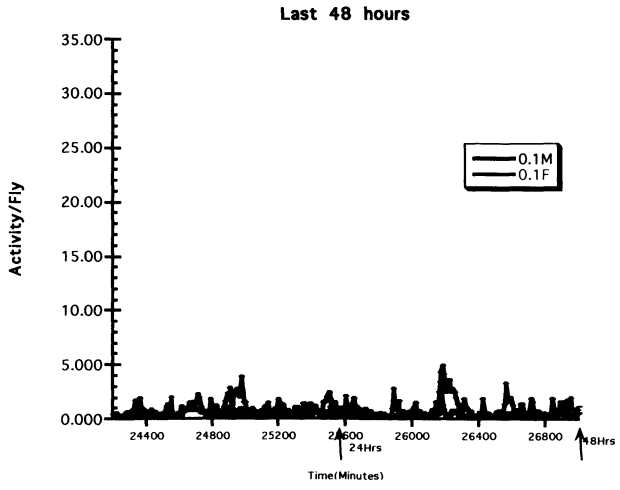


Figure 20

Age dependent changes in average mobility of flies maintained on 0.2N food level. The activity of first 48 hours compared with last 48 hours shows an increase in the activity in the last 48 hours

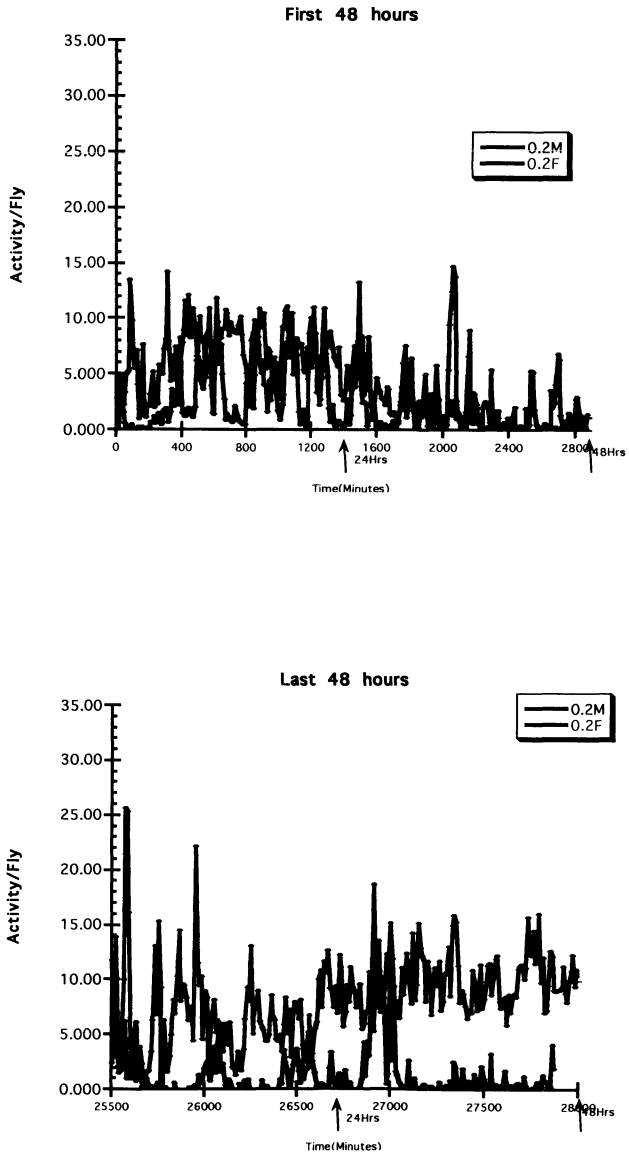


Figure 21

Male flies switched from high caloric 3.0N food level to low caloric 0.5N food level on Day 10. There is an increase in the average activity of flies after the dietary switch

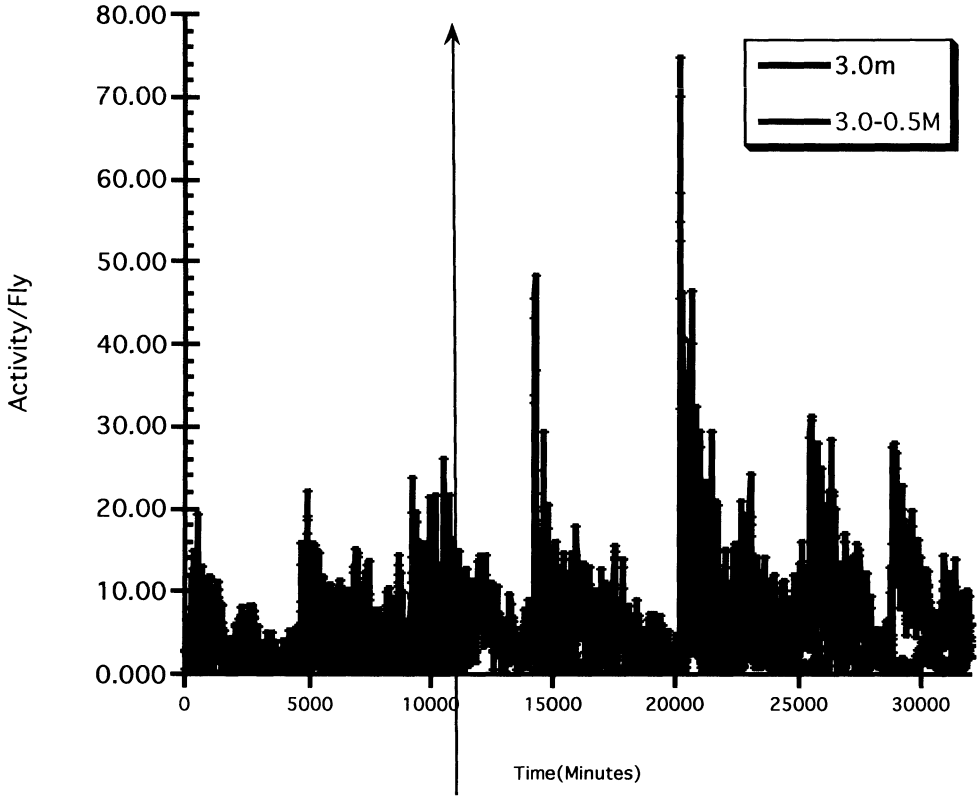


Figure 22

Female flies switched from high caloric 3.0N food level to low caloric 0.5N food level on Day 10 show a delayed response and a late increase in the average physical activity

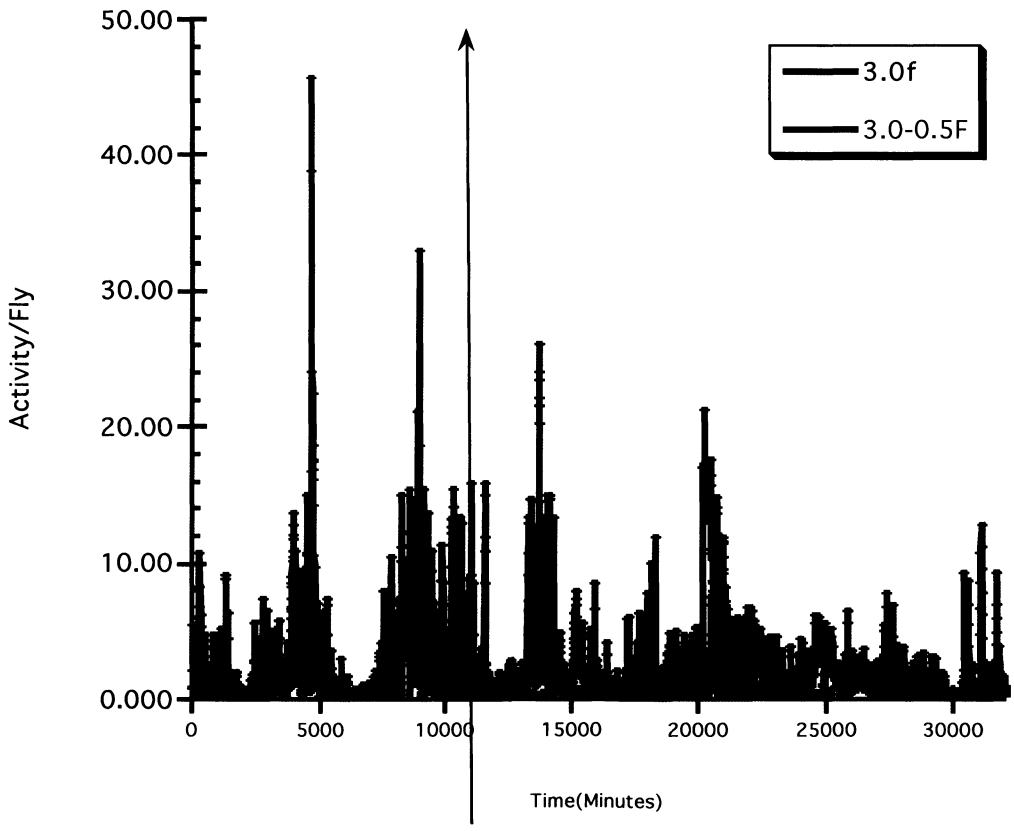


Figure 23

Male flies switched from low caloric 0.5N food level to high caloric 3.0N food level on Day 10. There is a decrease in the average activity of flies after the dietary switch. These flies show a higher activity level than flies maintained on high caloric diet from Day 0

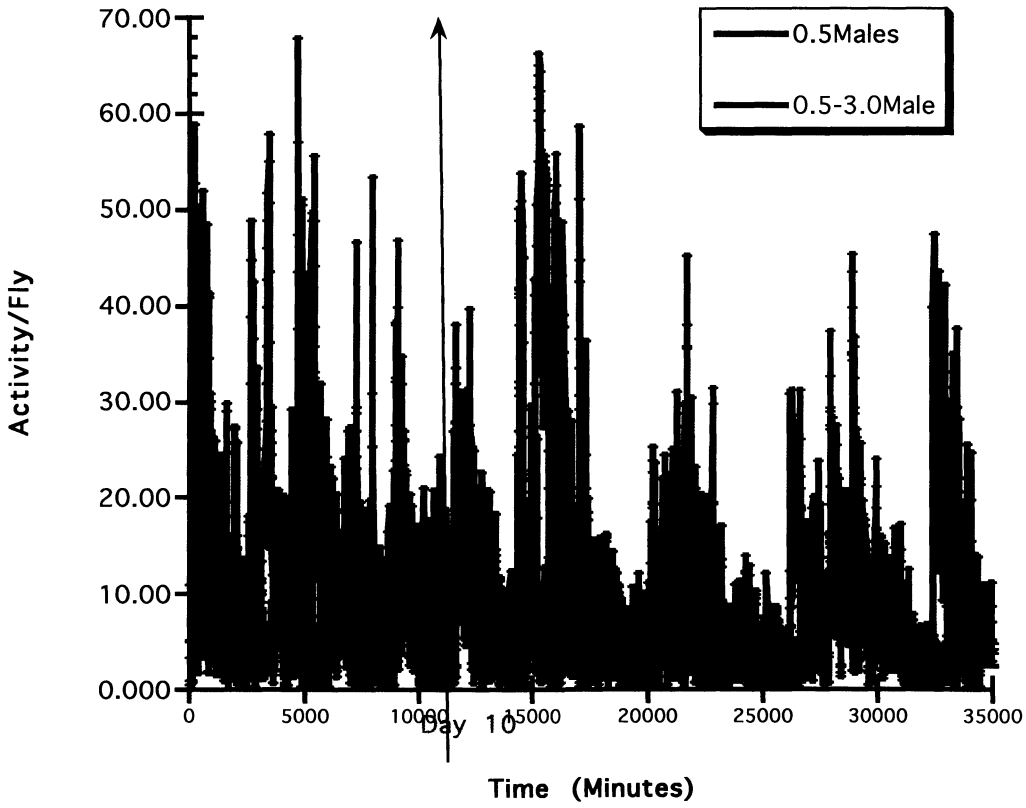


Figure 24

Female flies switched from low caloric 0.5N food level to high caloric 3.0N food level on Day 10. There is a decrease in the average activity of flies after the dietary switch

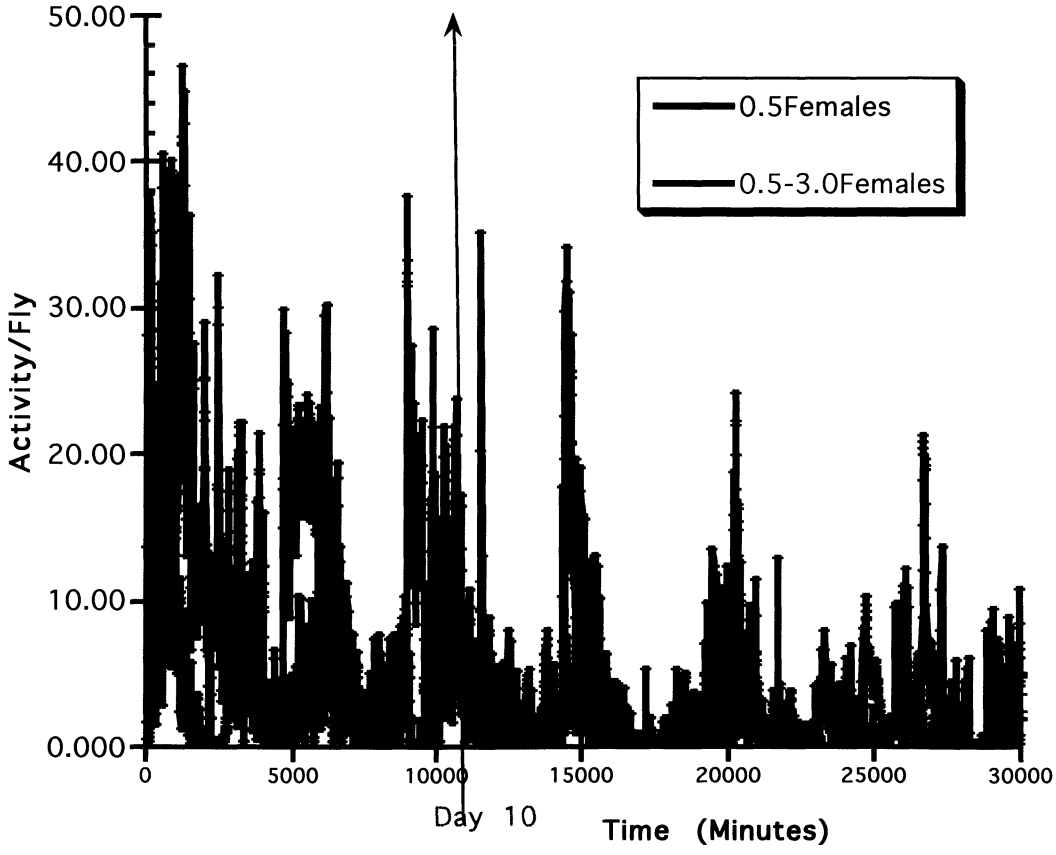


Figure 25

Mean of average activities of male flies after switch from low to high and high to low caloric diet. Flies seem to retaining a memory or show delayed activity response corresponding to the dietary food level before switch

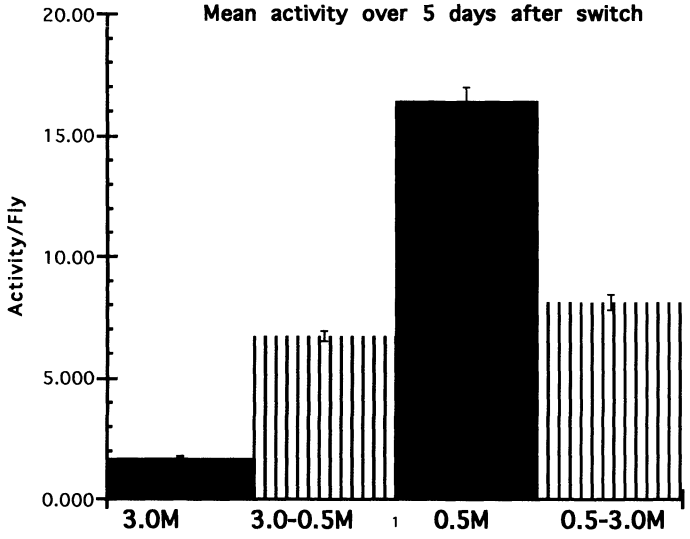
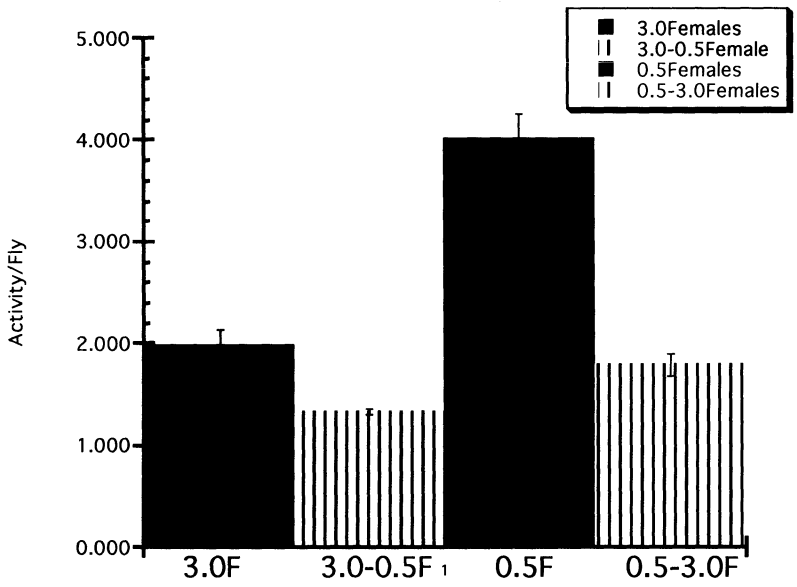


Figure 26

Mean of average activities of female flies after switch from low to high and high to low caloric diet. Flies switched from high to low caloric food appear to have reduced activity than the activity corresponding to low caloric levels



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